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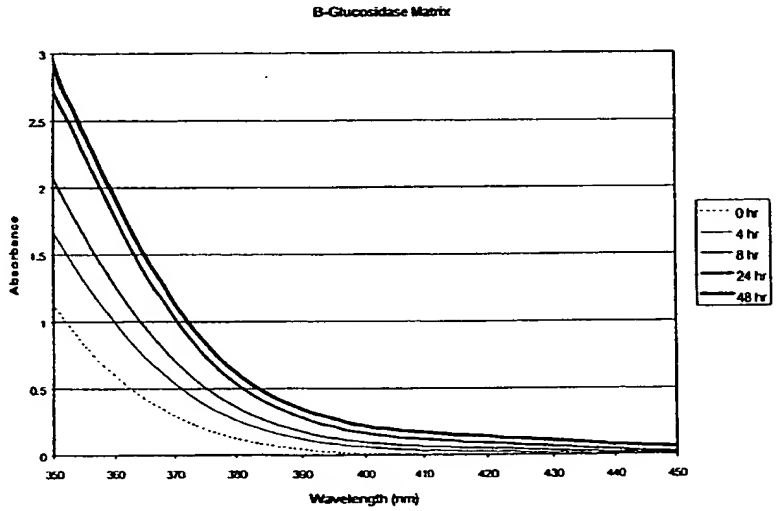
[Continued on next page]

(54) Title: MATRICES FOR DRUG DELIVERY AND METHODS FOR MAKING AND USING THE SAME



Enzymatic activity assay for a matrix containing β-Glucosidase.

(57) Abstract: In one aspect, biocompatible matrices such as sol-gels encapsulating a reaction center may be administered to a subject for conversion of prodrugs into biologically active agents. In certain embodiments, the biocompatible matrices of the present invention are sol-gels. In one embodiment, the enzyme L-amino acid decarboxylase is encapsulated and implanted in the brain to convert L-dopa to dopamine for treatment of Parkinson's disease.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat' l Application No
PCT/US 00/03754

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/03754

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-61,63,66 and 104-138
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although said claims are directed to a therapeutic method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition (Rule 39.1(iv)PCT).

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interna[illegible]al Application No
PCT/US 00/03754

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE CAS [Online] ANDERSON W A ET AL: "Development of a multienzyme reactor for dopamine synthesis: II. Reactor engineering and simulation" retrieved from CAS, accession no. 116-172334 XP002901041 abstract & BIOTECHNOL. BIOENG., vol. 39, no. 7, 1992, pages 781-789, ISSN: 0006-3592	1-143
A	DATABASE WPI Section Ch, Week 197951 Derwent Publications Ltd., London, GB; Class A96, AN 1979-91656B XP002901042 & JP 54 143588 A (UNITIKA LTD), 8 November 1979 (1979-11-08) abstract	1-143
A	DATABASE WPI Section Ch, Week 199318 Derwent Publications Ltd., London, GB; Class A96, AN 1993-149780 XP002901043 & JP 05 087811 A (KONICA CORP), 6 April 1993 (1993-04-06) abstract	1-143
A	EP 0 642 799 A (HOECHST SA LAB) 15 March 1995 (1995-03-15) abstract; claims	1-143

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48 C12N11/14 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 795 334 A (HOECHST AG) 17 September 1997 (1997-09-17) abstract page 6, line 9 - line 34 page 6, line 53 - line 58; claims 1,6-8 ---	1-143
Y	DATABASE CAS [Online] ECKERT-LILL ET AL: "Immobilized drugs: phenol and benzoic acid derivatives chemiabsorbed on silica. I. Preparation of chemiabsorbates" retrieved from CAS, accession no. 110-160327 XP002901040 abstract & ACTA PHARM. JUGOSL., vol. 38, no. 4, 1988, pages 373-379, ISSN: 0001-6667 --- -/--	1-143

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 May 2000	14 07 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Krenn

Form PCT/ISA/210 (second sheet) (July 1992)

Entrapment of Penicillinase (Type I-from Bacillus cereus, lyophilized powder containing approx. 10% protein, Sigma) was performed as outlined above, using 50mM pH 6.5 phosphate buffer. Penicillinase activity was determined using 100mL of a 3mM solution of Penicillin G (Benzylpenicillin, sodium salt, Sigma) in buffer. 2mL aliquots of the reaction solution were removed for assay and their UV-Vis spectra recorded.

c) Tyrosinase

Entrapment of Tyrosinase (from mushroom, Sigma) was performed as outlined above, with 50mM pH 6.5 phosphate buffer solution. Tyrosinase activity assays were performed using 0.3mM L-tyrosine (Aldrich, 99+%) solution in buffer.

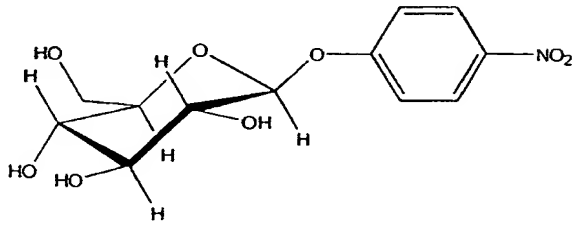
d) Tyrosine Decarboxylase

Entrapment of Tyrosine Decarboxylase (from Streptococcus faecalis, Fluka) was performed using 50mM pH 5.5 acetate buffer and was accomplished by the method outlined above. The activity assay was accomplished using a 50:50 mixture of 2.5mM solution of L-tyrosine (Aldrich, 99+%) in buffer and buffer. Total reaction volume for this assay was either 40mL (19.5h data reported herein) or 100mL (all other data reported). 2mL aliquots of reaction mixture were removed from the reaction vessel for assay. The method used for this assay was addition of 1mL of a 1M K_2CO_3 solution to the 2mL aliquot, followed by mixing. To this was added 2 drops of a solution of picrylsulfonic acid (5% w/v aquesous solution of 2,4,6-trinitrobenzenesulfonic acid, Sigma). The mixture was mixed well. 2mL of toluene were added to this mixture, the layers shaken well, and centrifuged. The toluene layer was removed and its UV-Vis spectrum collected. Where applicable, a 0.1mM solution of pyridoxal-5-phosphate monohydrate (98%, Aldrich) in buffer was substituted for the buffer solution in the assay mixture. Assays performed in the presence of cofactor were carried out in foil-covered reaction vessels due to the sensitivity of pyridoxal-5-phosphate to light.

3. Results of Enzyme Encapsulation and Assays

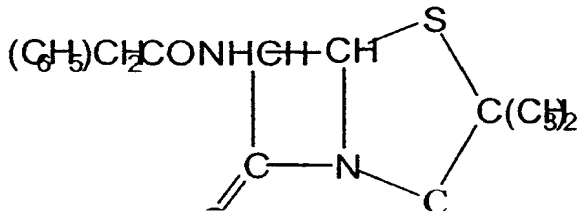
a) b-Glucosidase entrapment yielded active matrices which were assayed using the synthetic substrate para-nitrophenyl-b-D-glucopyranoside, shown below. Enzymatic activity of matrix composites on the synthetic substrate results in cleavage of the

glucosidic bond producing a bathochromic shift in the spectral band. This shift permits monitoring of the cleavage process, as illustrated in Figure 1.



The synthetic substrate para-nitrophenyl-b-D-glucopyranoside.

b) Penicillinase entrapment provided active matrices which were assayed using the sodium salt of the synthetic substrate benzylpenicillin, shown below. Conversion of penicillin to penicilloic acid via rupture of the β-lactam ring may be monitored spectrophotometrically, as shown in Figure 2.



Substrate used for penicillinase activity assay, sodium benzylpenicillin.

Reproducibility of the measurements done for penicillinase was checked by performing activity assay multiple times for the same matrix. As shown in Figure 3(a), good agreement is observed for multiple assays performed over six consecutive days. Likewise, running multiple matrices from the same preparation to check reproducibility of the matrix entrapment shows good agreement, as seen in Figure 3(b).

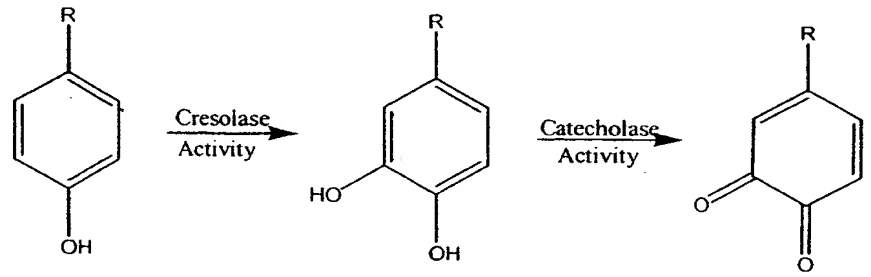
Loading studies utilizing penicillinase matrices were performed in which the enzyme concentration was varied over a wide range to dtermine the optimal enzyme concentration. The bar graph shown in Figure 4 shows the effects of varying enzyme concentration on the activity of the matrix. The highest percentage of activity observed as a function of enzyme entrapped within the matrix (selected from the five compositions analyzed) occurs for the lowest concentration of enzyme examined, as shown in Figure 5.

Surface area effects were also examined utilizing penicillinase matrices. Ten identical monoliths were prepared and aged simultaneously. Five of these were assayed as whole monoliths (cast in 4.5 mL cuvettes) while another five matrices were coarsely crushed and then assayed. This qualitative examination of surface area effects revealed that an increase in surface area does result in an increase in the enzyme activity observed, as shown in Figures 6 and 7. It should be noted that there is no leaching of enzyme observed from either the whole or crushed matrices, as determined by soaking the matrices in buffer solution overnight and subsequently checking the activity of the soak solution. The reproducibility of the measurements for the assays shown in Figure 6 is quite reasonable, with the larger deviation in the crushed matrices attributable to the lack of control over particle size when breaking up the samples. Figure 7, showing the mean values for each measurement with error bars, emphasizes the greater relative activity of the crushed matrix samples.

The significant effect of changing surface area on the observed enzyme activity prompted further investigation. Control over total surface area was achieved by casting the sol containing penicillinase into varying numbers of cell culture plate wells (22.6 mm diameter). By varying the amount of sol cast into a given well, the total 4mL of material per matrix could be spread out over a number of wells and the disks cast in these wells could be recombined, after removal from the wells, for assay. Thus, the 4mL of sol that constitutes one matrix preparation could be cast into one or multiple wells to generate samples with known, varying surface areas. Surface area stated for a given matrix reflects the initial surface area of the gel when freshly cast, and does not attempt to correct for any shrinkage that occurred during aging. Figure 8(a) illustrates the difference in activity observed for matrices of varying surface areas. Figure 8(b) shows the activity as a percentage of the penicillinase activity used in the preparation of the matrices.

c) Following entrapment of tyrosinase, the bifunctional activity of this enzyme was found to complicate spectrophotometric assay of the matrix composite due to the variation in molar extinction coefficient of the different species, and possible retention within the matrix. Tyrosinase possesses both cresolase (conversion of phenols to diphenols) and catecholase activity (conversion of diphenols to the corresponding quinone), as shown below. However, a qualitative analysis shows the conversion of the natural substrate L-tyrosine to L-dopa, which then undergoes dehydrogenation to give dopaquinone. Dopaquinone is unstable in aqueous solutions and undergoes a Michealis

rearrangement to form, among other products, a number of melanin precursors which eventually polymerize to produce pigments. This complicated reaction may be followed qualitatively by monitoring a color change within the matrix. Although the L-tyrosine solution is, itself, colorless, the tyrosinase-containing matrices become noticeably darkened within one hour of contact with the substrate solution, suggesting formation of products with subsequent retention by the matrix. A solution of L-dopa in the presence of tyrosinase, likewise forms a gray-black precipitate.



Monophenol Polyphenol Quinone

d) Active Tyrosine Decarboxylase matrices were and assayed using L-tyrosine as substrate. A complicating factor in the assay of entrapped Tyrosine Decarboxylase was the unavailability of a direct spectrophotometric method due to the equivalent molar extinction coefficients of substrate and product. The observation necessitated the development of an indirect assay provided by Phan et. al., App. Biochem. Biotech. 8:127 (1983). The results of an active Tyrosine Decarboxylase composite assay are shown in Figure 9.

Longer aging times for Tyrosine Decarboxylase-containing matrices resulted in matrices for which no enzyme activity was observed without addition of cofactor. Addition of cofactor, pyridoxal-5-phosphate monohydrate (0.05mM), to the assay mixture restored activity of the entrapped enzyme to varying degrees depending on the aging of the monolith. Figure 10 shows activity assays for two 16 day old Tyrosine Decarboxylase-containing matrices, one without cofactor present and one with cofactor, and compares them to a matrix of the same composition assayed after aging 19h. The activity observed at 19h without cofactor and at 16d with cofactor present are nearly identical, whereas without the presence of cofactor little, if any, activity is noted. For matrices aged 50d a significant portion of the activity is retained in the presence in cofactor, although some loss of activity is observed.

B. Matrix Optimization Studies

1. Matrix Preparation

The general synthetic technique used for preparation of the silica sol was addition of appropriate aliquots of the organically substituted trimethoxysilane, tetramethyl orthosilicate and 4mM HCl solution to a 25 x 150mm test tube equipped with a stirbar. Total desired volume of sol was determined by the number of matrices to be prepared. The $RSi(OCH_3)_3$ and TMOS reagents were combined in appropriate ratios to yield the desired compositions.

Reagent	Source
Tetramethylorthosilicate (TMOS)	Aldrich, 99+%
Methyltrimethoxysilane (MTMS)	Aldrich, 98%
Ethyltrimethoxysilane (ETMS)	Aldrich, 97+%
Trimethoxypropylsilane (TMPS)	Aldrich, 98%
iso-Butyltrimethoxysilane (i-BTMS)	Aldrich, 97%
n-Butyltrimethoxysilane (n-BTMS)	United Chemical Technologies, 95.3%
Phenyltrimethoxysilane (PTMS)	Aldrich, 97%

As with 100% TMOS matrices, the mixture is stirred until homogeneous (approximately 15 minutes). The test tube containing the sol is then transferred to an ice bath and allowed to cool for 10 minutes. A 2mL aliquot of sol is then transferred to another chilled test tube in an ice bath and stirred. To this sol, 1mL of chilled buffer solution (appropriate to the enzyme to be entrapped) is added, and stirred for ca. 10s, followed by addition of 1mL of chilled, buffered solution containing the desired enzyme. The sol is swirled briefly, and then pipetted into a 4.5 mL polystyrene cuvette (cell culture dishes were also used for surface area study matrices). The cuvette opening is sealed with Parafilm following gel formation (cell culture dish covers were used for surface area study matrices). The gel is then allowed to age in the sealed container for a period of time ranging from 14 to 50 days or more at temperatures ranging from 4°C to room temperature.

2. Enzyme Encapsulation and Assays

Entrapment of Penicillinase (Type I from Bacillus cereus, lyophilized powder containing approx. 10% protein, Sigma) was performed as outlined above, using 50mM pH 6.5 phosphate buffer. Penicillinase activity was determined using 100mL of a 3mM solution of Penicillin G (Benzylpenicillin, sodium salt, Sigma) in buffer. 2mL aliquots of the reaction solution were removed for assay and their UV-Vis spectra recorded.

3. Results of Enzyme Encapsulation and Assays

Initial examination of which matrix compositions provided matrices suitable for the purposes of this study excluded the n-butyltrimethoxysilane composition, as well as some of the higher ratios of other $RSi(OCH_3)_3$ precursors, due to the failure of these compositions to form a gel that was appropriate for our intended uses. Compositions examined, and their reactivity relative to 100% TMOS matrices are shown in Table 1.

Table 1. Activity for given compositi ns relative to 100% TMOS.

	Composition	Relative Activity
MTMS : TMOS	10% MTMS : 90% TMOS	108%
	20% MTMS : 80% TMOS	82%
	30% MTMS : 70% TMOS	92%
	40% MTMS : 60% TMOS	104%
	50% MTMS : 50% TMOS	112%*
ETMS : TMOS	10% ETMS : 90% TMOS	99%
	20% ETMS : 80% TMOS	93%
	30% ETMS : 70% TMOS	99%
TMPS : TMOS	10% TMPS : 90% TMOS	100%
	20% TMPS : 80% TMOS	80%
i-BTMS : TMOS	10% i-BTMS : 90% TMOs	90%
	20% i-BTMS : 80% TMOs	75%
PTMS : TMOS	10% PTMS : 90% TMOS	94%

* Indicates a composition in which slight enzyme leaching is observed at the time of the assay. If insufficient aging is allowed, enzyme leaching is observed for methyltrimethoxysilane composition with MTMS content greater than 20%.

In addition, it was observed that as the matrices age the relative activity of the MTMS-containing matrices with respect to 100% TMOS drops. When matrices from the same preparation are assayed after aging 104 days at 4°C, the relative activity observed is shown in Table 2.

Table 2. Enzyme activity relative to 100% TMOS for varying MTMS-containing matrices aged 104 days.

	Composition	Relative Activity
MTMS : TMOS	10% MTMS : 90% TMOS	85%
	20% MTMS : 80% TMOS	78%
	30% MTMS : 70% TMOS	85%
	40% MTMS : 60% TMOS	85%
	50% MTMS : 50% TMOS	103%*

*Indicates a composition in which no enzyme leaching is observed at the time of this assay, although leaching is observed for shorter aging time.

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference and set forth in its entirety herein. In case of conflict, the present application, including any definitions herein, will control. In addition to the foregoing materials, the practice of the present invention may employ in part, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning a Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); U.S. Patent No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription and Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors for Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods in Enzymology, Vols. 154 and 155

(Wu et al. eds.), Immunochemical Methods in Cell-and Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986), all of which references are hereby incorporated by reference to the same extent as the other references specified herein.

The specification and examples should be considered exemplary only with the true scope and spirt of the invention suggested by the following claims.

WHAT IS CLAIMED IS:

1. A method for producing a biologically active agent from a prodrug, comprising:
   a. encapsulating a first cell-free reaction center in a biocompatible matrix; and
   b. administering said biocompatible matrix to a subject;
wherein said biocompatible matrix comprises an inorganic-based sol-gel matrix and wherein said first reaction center converts a first prodrug into a first biologically active agent in said subject.

2. The method of claim 1, wherein said biocompatible matrix comprises a silica-based sol-gel matrix.

3. The method of claim 2, wherein said first reaction center comprises one of the following: an enzyme, an antibody or a catalytic antibody.

4. The method of claim 2, wherein said biocompatible matrix encapsulates at least one additive.

5. The method of claim 2, wherein said first reaction center comprises L-amino acid decarboxylase.

6. The method of claim 5, wherein said first prodrug comprises L-dopa and said first biologically active agent comprises dopamine.

7. The method of claim 2, wherein said first reaction center comprises L-tyrosine decarboxylase.

8. The method of claim 7, wherein said first prodrug comprises L-dopa and said first biologically active agent comprises dopamine.

9. The method of claim 2, further comprising encapsulating a second reaction center in said biocompatible matrix before administering said biocompatible matrix to said subject.

10. The method of claim 9, wherein said first biologically active agent produced by said first reaction center from said first prodrug is a second prodrug for said second reaction center, and wherein said second reaction center produces a second biologically active agent that differs from said first biologically active agent.

11. The method of claim 12, wherein said first reaction center comprises tyrosine monooxygenase, and said second reaction center is one of the following: L-amino acid decarboxylase or L-tyrosine decarboxylase.

12. The method of claim 11, wherein said first prodrug comprises tyrosine, said first biologically active agent and said second prodrug comprises L-dopa, and said second biologically active agent comprises dopamine.

13. The method of claims 4, 5, 6, 7, 8, 11 or 12, wherein administering said biocompatible matrix comprises administering said biocompatible matrix to a region of the brain of said subject.

14. The method of claim 13, wherein said region of said brain of said subject is one of the following: basal ganglia, substantia nigra or striatum.

15. The method of claim 2, wherein said biocompatible matrix is prepared from at least one type of oxysilane.

16. The method of claim 15, wherein said biocompatible matrix is prepared from more than one type of oxysilane.

17. The method of claim 15, wherein said biocompatible matrix is prepared from at least one type of inorganic oxide and at least one type of oxysilane.

18. The method of claim 15 or 16, wherein said type of oxysilane has at least one non-hydrolizable substituent.

19. The method of claim 2, wherein said biocompatible matrix consists essentially of siloxane.

20. The method of claim 2, wherein said biocompatible matrix comprises siloxane.

21. The method of claim 2, wherein administering said biocompatible matrix comprises surgical implantation.

22. The method of claim 2, further comprising administering said first prodrug to said subject.

23. The method of claim 2, wherein said first prodrug comprises an exogenous prodrug.

24. The method of claim 2, wherein said first prodrug comprises an endogenous prodrug.

25. The method of claim 2, wherein said first reaction center comprises an enzyme or antibody that is xenogeneic to said subject.

26. The method of claim 3, wherein the ratio of Km (nonencapsulated) to Km (encapsulated) for said first reaction center is greater than or equal to one.

27. The method of claim 3, wherein the ratio of Km (nonencapsulated) to Km (encapsulated) for said first reaction center is less than or equal to one.

28. The method of claim 2, wherein said first reaction center comprises more than one weight percent of said biocompatible matrix.

29. The method of claim 2, wherein said first reaction center comprises less than one weight percent of said biocompatible matrix.

30. The method of claim 29, wherein said first reaction center comprises more than five weight percent of said biocompatible matrix.

31. The method of claim 31, wherein said first reaction center comprises more than ten weight percent of said biocompatible matrix.

32. The method of claim 2, wherein said first reaction center is attached to said biocompatible matrix.

33. The method of claim 2, wherein said biocompatible matrix is immunoisolatory.

34. The method of claim 2, wherein administering said biocompatible matrix comprises parenteral administration.

35. The method of claim 2, wherein administering said biocompatible matrix comprises systemic administration.

36. The method of claim 2, wherein treatment of said subject by said method results in long-term, stable production of said first biologically active agent in said subject.

37. The method of claim 22, wherein said first prodrug is administered to said subject on at least more than one occasion.

38. The method of claim 2, wherein said first biologically active agent is cytotoxic.

39. The method of claim 38, wherein said biocompatible matrix is implanted in proximity to a neoplasm.

40. The method of claim 2, wherein said first reaction center does not leach significantly from said biocompatible matrix.

41. The method of claim 2, wherein said biocompatible matrix comprises a xero-gel.

42. The method of claim 15, wherein said oxysilane is one of the following: TMOS or TEOS.

43. The method of claim 3, wherein said biocompatible matrix causes prodrug activation.

44. The method of claim 2, wherein said first prodrug is a deleterious agent to said subject and said first biologically active agent is less deleterious to said subject than said first prodrug.

45. The method of claim 44, wherein said first prodrug is an agent to which said subject is capable of becoming addicted, and wherein said subject is less capable of becoming addicted to said first biologically active agent.

46. The method of claim 45, wherein said first prodrug is one of the following: ethanol or cocaine.

47. The method of claim 2, wherein said first prodrug is one of the following: L-phenylalanine, noradrenalin, norepinephrine, histadine, histamine, 1-methylhistamine, glutumate, GABA or serine.

48. The method of claim 2, wherein said subject is human.

49. The method of claim 2, wherein said subject receives a therapeutically effective amount of said biocompatible matrix and said first prodrug.

50. The method of claim 23, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index of treatment using said first prodrug alone is about five or more.

51. The method of claim 50, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index of treatment using said first prodrug alone is about ten or more.

52. The method of claim 51, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index of treatment using said first prodrug alone is at least about one hundred.

53. The method of claim 37, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index

of treatment using the biologically active agent of said first prodrug alone is at about five or more.

54. The method of claim 53, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index of treatment using the biologically active agent of said first prodrug alone is at about ten or more.

55. The method of claim 51, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index of treatment using the biologically active agent of said first prodrug alone is at least about one hundred.

56. The method of claim 2, wherein said first biologically active agent comprises a neutrophic factor.

57. The method of claim 2, wherein said first biologically active agent comprises a type selected from the group consisting of anti-angiogenesis factors, antiinfectives; antibiotics agents; antiviral agents; analgesics; anorexics; antihelmintics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antidiuretic agents; antidiarrheals; antihistamines; antiinflammatory agents; antimigraine preparations; antinauseants; antineoplastics; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics, antispasmodics; anticholinergics; sympathomimetics; xanthine derivatives; cardiovascular preparations; calcium channel blockers; beta-blockers; antiarrhythmics; antihypertensives; catecholamines; diuretics; vasodilators; central nervous system stimulants; cough preparations; cold preparations; decongestants; growth factors, hormones; steroids,; corticosteroids; hypnotics; immunosuppressives; muscle relaxants; parasympatholytics; psychostimulants; sedatives; tranquilizers; proteins; polysaccharides; glycoproteins; lipoproteins; interferons; cytokines; chemotherapeutic agents; anti-neoplastics, antibiotics, anti-virals, anti-fungals, anti-inflammatories, anticoagulants, lymphokines, and antigenic materials.

58. The method of claim 3, wherein said first reaction center comprises an enzyme that is a member of a class selected from the group consisting of oxidoreductases; transferases; hydrolases; isomerases; and ligases.

59. The method of claim 2, wherein said first reaction center replaces, augments or supplements some endogenous biological activity in said subject.

60. The method of claim 59, wherein said first reaction center comprises an enzyme in which said subject is deficient.

61. The method of claim 60, wherein said first reaction center is one of the following: glucocerebrosidase; α-1,4 - glucosidase; α-galactosidase; α-L-iduronidase; β-glucuronidase; aminolaevulinate dehydratase; bilirubin oxidase; catalase; fibrinolysin; glutaminase; hemoglobin; heparinase; L-arginine ureahydrolase (A1); arginase; liver microsomal enzymes; phenylalanine ammonia lyase; streptokinase; superoxide dismutase; terrilythin; tyrosinase; UDP glucuronyl transferase; urea cycle enzymes; urease; uricase; or urokinase.

62. A method of toxicology testing, comprising:

a. encapsulating at least one reaction center in a silica-based sol-gel matrix;

b. interacting a compound with said matrix; and,

c. evaluating for any products of said compound resulting from conversion of said compound by said reaction center,

wherein production of any cytotoxic or mutagenic products indicates that said compound may be toxic to a subject upon administration.

63. The method of claim 62, wherein said reaction center comprises an enzyme that is located in the liver of a mammal.

64. The method of claim 62, wherein said reaction center is an enzyme prepared by recombinant methods.

65. The method of claim 62, wherein said reaction center is cell-free.

66. The method of claim 63, wherein said mammal is one of the following: a pig or a human.

67. A biocompatible matrix for treatment, comprising:

a. a inorganic-based sol-gel matrix that is biocompatible; and,

b. a first cell-free reaction center encapsulated in said matrix,

wherein said first reaction center, after administration of said matrix to a subject, produces a therapeutically effective amount of a first biologically active agent from a first prodrug in said subject.

68. The biocompatible matrix of claim 67, wherein said biocompatible matrix comprises a silica-based sol-gel matrix.

69. The biocompatible matrix of claim 67, wherein said first reaction center comprises one of the following: an enzyme, an antibody or a catalytic antibody.

70. The biocompatible matrix of claim 68, wherein said first reaction center comprises one of the following: L-amino acid decarboxylase or L-tyrosine decarboxylase.

71. The biocompatible matrix of claim 71, wherein said first reaction center comprises L-amino acid decarboxylase, said first prodrug comprises L-dopa, and said first biologically active agent comprises dopamine.

72. The biocompatible matrix of claim 68, wherein said biocompatible matrix further comprises a second reaction center.

73. The biocompatible matrix of claim 72, wherein said first biologically active agent produced by said first reaction center from said first prodrug is a second prodrug for said second reaction center, and wherein said second reaction center produces a second biologically active agent that differs from said first biologically active agent.

74. The biocompatible matrix of claim 73, wherein said first reaction center comprises tyrosine monooxygenase, and said second reaction center is one of the following: L-amino acid decarboxylase or L-tyrosine decarboxylase.

75. The biocompatible matrix of claims 70, 71 or 74, wherein administering said biocompatible matrix comprises administering said biocompatible matrix to a region of the brain of said subject.

76. The biocompatible matrix of claim 75, wherein said region of said brain of said subject is one of the following: basal ganglia, substantia nigra or striatum.

77. The biocompatible matrix of claim 68, wherein said biocompatible matrix is prepared from at least one type of oxysilane.

78. The biocompatible matrix of claim 68, wherein said biocompatible matrix is siloxane.

79. The biocompatible matrix of claim 77, wherein said type of oxysilane has at least one non-hydrolizable substituent.

80. The biocompatible matrix of claim 68, wherein said first prodrug is exogenous to said subject.

81. The biocompatible matrix of claim 67, wherein said first prodrug is endogenous to said subject.

82. The biocompatible matrix of claim 68, wherein said first reaction center comprises an enzyme that is xenogeneic to said subject.

83. The biocompatible matrix of claim 69, wherein the ratio of K_m (nonencapsulated) to K_m (encapsulated) for said first reaction center is greater than or equal to one.

84. The biocompatible matrix of claim 67, wherein said first reaction center comprises more than one weight percent of said biocompatible matrix.

85. The biocompatible matrix of claim 82, wherein said xenogeneic enzyme comprises more than five weight percent of said biocompatible matrix.

86. The biocompatible matrix of claim 68, wherein said first reaction center comprises more than ten weight percent of said biocompatible matrix.

87. The biocompatible matrix of claim 68, wherein said biocompatible matrix is immunoisolatory.

88. The biocompatible matrix of claim 68, wherein said biocompatible matrix is capable of long-term, stable production of said first biologically active agent in said subject.

89. The biocompatible matrix of claim 69, wherein said first biologically active agent is cytotoxic.

90. The biocompatible matrix of claim 67, wherein said first reaction center does not leach significantly from said biocompatible matrix after administration.

91. The biocompatible matrix of claim 68, wherein said biocompatible matrix comprises a xero-gel.

92. The biocompatible matrix of claim 77, wherein said oxysilane is one of the following: TMOS or TEOS.

93. The biocompatible matrix of claim 68, wherein said first prodrug is a deleterious agent to said subject and said first biologically active agent is less deleterious to said subject than said first prodrug..

94. The biocompatible matrix of claim 67, wherein said first prodrug is an agent to which said subject is capable of becoming addicted, and wherein said subject is less capable of becoming addicted to said first biologically active agent.

95. The biocompatible matrix of claim 68, wherein said first prodrug is one of the following: L-phenylalanine, noradrenalin, norepinephrine, histadine, histamine, l-methylhistamine, glutumate, GABA or serine.

96. The biocompatible matrix of claim 80, wherein the ratio of the therapeutic index of treatment using said first prodrug and said first biocompatible matrix over the therapeutic index of treatment using said first prodrug alone is about five or more.

97. The biocompatible matrix of claim 88, wherein the ratio of the therapeutic index of treatment using said first prodrug and said first biocompatible matrix over the therapeutic index of treatment using said first prodrug alone is at least about one hundred.

98. The biocompatible matrix of claim 80, wherein the ratio of the therapeutic index of treatment using said first prodrug and said biocompatible matrix over the therapeutic index of treatment using the biologically active agent of said first prodrug alone is at least about ten or more.

99. The biocompatible matrix of claim 80, wherein said first biologically active agent comprises a type selected from the group consisting of anti-angiogenesis factors, antiinfectives; antibiotics agents; antiviral agents; analgesics; anorexics; antihelmintics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antidiuretic agents; antidiarrheals; antihistamines; antiinflammatory agents; antimigraine preparations; antinauseants; antineoplastics; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics, antispasmodics; anticholinergics; sympathomimetics; xanthine derivatives; cardiovascular preparations; calcium channel blockers; beta-blockers; antiarrhythmics; antihypertensives; catecholamines; diuretics; vasodilators; central nervous system stimulants; cough preparations; cold preparations; decongestants; growth factors, hormones; steroids,; corticosteroids; hypnotics; immunosuppressives; muscle relaxants; parasympatholytics; psychostimulants; sedatives; tranquilizers; proteins; polysaccharides; glycoproteins; lipoproteins; interferons; cytokines; chemotherapeutic agents;

anti-neoplastics, antibiotics, anti-virals, anti-fungals, anti-inflammatories, anticoagulants, lymphokines, and antigenic materials.

100. The biocompatible matrix of claim 68, wherein said first reaction center comprises an enzyme that is member of a class of one of the following: oxidoreductases; transferases; hydrolases; isomerases; or ligases.

101. The biocompatible matrix of claim 67, wherein said first reaction center replaces, augments or supplements some endogenous biological activity in said subject.

102. The biocompatible matrix of claim 68, wherein said first reaction center comprises an enzyme in which said subject is deficient.

103. The biocompatible matrix of claim 102, wherein said first reaction center is one of the following: glucocerebrosidase; α-1,4 - glucosidase; α-galactosidase; α-L-iduronidase; β-glucuronidase; aminolaevulinate dehydratase; bilirubin oxidase; catalase; fibrinolysin; glutaminase; hemoglobin; heparinase; L-arginine ureahydrolase (A1); arginase; liver microsomal enzymes; phenylalanine ammonia lyase; streptokinase; superoxide dismutase; terrilythin; tyrosinase; UDP glucuronyl transferase; urea cycle enzymes; urease; uricase; or urokinase.

104. A biologically active agent produced by a process comprising:

a. encapsulating a first cell-free reaction center in a biocompatible matrix; and

b. administering said biocompatible matrix to a subject;

wherein said biocompatible matrix comprises an inorganic-based sol-gel matrix, and wherein said biologically active agent is produced by said first reaction center from a first prodrug in said subject.

105. The biologically active agent of claim 104, wherein said biocompatible matrix comprises a silica-based sol-gel matrix.

106. The biologically active agent of claim 105, wherein said first reaction center comprises one of the following: an enzyme, an antibody or a catalytic antibody.

107. The biologically active agent of claim 106, wherein said first reaction center is one of the following: L-amino acid decarboxylase, L-tyrosine decarboxylase or tyrosine monooxygenase,

108. The biologically active agent of claim 105, further comprising encapsulating a second reaction center in said biocompatible matrix before administering said biocompatible matrix to said subject.

109. The biologically active agent of claim 107, wherein administering said biocompatible matrix comprises administering said biocompatible matrix to one of the following regions of the brain: basal ganglia, substantia nigra or striatum.

110. The biologically active agent of claim 105, further comprising preparing said biocompatible matrix from at least one type of oxysilane at substantially the same time as said encapsulating of said first reaction center.

111. The biologically active agent of claim 105, wherein said biocompatible matrix consists essentially of siloxane.

112. The biologically active agent of claim 105, wherein administering said biocompatible matrix to a subject comprises surgical implantation.

113. The biologically active agent of claim 110, further comprising administering said first prodrug to said subject.

114. The biologically active agent of claim 105, wherein said first prodrug comprises a prodrug exogenus to said subject.

115. The biologically active agent of claim 105, wherein said process results in long-term, stable production of said biologically active agent in said subject.

116. The biologically active agent of claim 114, wherein said first prodrug is administered to said subject on at least more than one occasion.

117. The biologically active agent of claim 105, wherein said first prodrug is a deleterious agent to said subject and said first biologically active agent is less deleterious to said subject than said first prodrug..

118. The biologically active agent of claim 105, wherein said subject is human.

119. The biologically active agent of claim 105, wherein said biologically active agent comprises a type selected from the group consisting of anti-angiogenesis factors, antiinfectives; antibiotics agents; antiviral agents; analgesics; anorexics; antihelmintics; antiarthritics; antiasthmatic agents; anticonvulsants; antidepressants; antidiuretic agents; antidiarrheals; antihistamines; antiinflammatory agents; antimigraine preparations; antinauseants; antineoplastics; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics, antispasmodics; anticholinergics; sympathomimetics; xanthine derivatives; cardiovascular preparations; calcium channel blockers; beta-blockers; antiarrhythmics; antihypertensives; catecholamines; diuretics; vasodilators; central nervous system stimulants; cough preparations; cold preparations; decongestants; growth factors, hormones; steroids,; corticosteroids; hypnotics; immunosuppressives; muscle relaxants; parasympatholytics; psychostimulants; sedatives; tranquilizers; proteins; polysaccharides; glycoproteins; lipoproteins; interferons; cytokines; chemotherapeutic agents; anti-neoplastics, antibiotics, anti-virals, anti-fungals, anti-inflammatories, anticoagulants, lymphokines, and antigenic materials.

120. The biologically active agent of claim 105, wherein said first reaction center comprises an enzyme that is member of a class selected from the group consisting of oxidoreductases; transferases; hydrolases; isomerases; and ligases.

121. The biologically active agent of claim 111, wherein said first reaction center comprises an enzyme in which said subject is deficient.

122. A tissue assist device, comprising:

a. a inorganic-based sol-gel matrix that is biocompatible; and,

b. a first reaction center encapsulated in said matrix,

wherein upon placing said biocompatible matrix in contact with fluids of a subject, said first reaction center converts a first prodrug into a first biologically active agent, and wherein said first reaction center provides a biological function characteristic of tissue of said subject.

123. The device of claim 122, wherein said biocompatible matrix comprises a silica-based sol-gel matrix.

124. The device of claim 123, wherein said tissue is an organ.

125. The device of claim 124, wherein said organ is a liver.

126. The device of claim 125, wherein said first reaction center is one of the following: cytochrome P-450, hepatocytes or Kupffer cells.

127. The device of claim 124, wherein said first prodrug is endogenous to said subject and is more deleterious to said subject than said first biologically active agent.

128. The device of claim 123, wherein said fluid is blood of said subject.

129. The device of claim 125, wherein said first reaction center is xenogeneic.

130. The device of claim 123, wherein said contact occurs extracorporeal to said subject.

131. The device of claim 123, wherein said tissue of said subject is deficient in converting said first prodrug into said first biologically active agent.

132. A kit for treatment of a subject, comprising:

a. a inorganic-based sol-gel matrix that is biocompatible; and,

b. a first cell-free reaction center encapsulated in said matrix,

wherein said first reaction center, after administration of said matrix to a subject, produces a therapeutically effective amount of a first biologically active agent from a first prodrug in said subject.

133. The kit of claim 132, wherein said biocompatible matrix comprises a silica-based sol-gel matrix.

134. The kit of claim 133, further comprising instructions for treatment of said subject using said kit.

135. The kit of claim 133, further comprising one or more doses of said first prodrug for administration to said subject.

136. The kit of claim 135, wherein said dose of said first prodrug is formulated for controlled release of said first prodrug upon administration to said subject.

137. A method of treatment of a subject, comprising:

a. a step for encapsulating a first cell-free reaction center in a biocompatible matrix; and

b. a step for administering said biocompatible matrix to a subject;

wherein said biocompatible matrix comprises a silica-based sol-gel matrix, and wherein said first reaction center converts a first prodrug into a first biologically active agent in said subject.

138. The method of treatment of claim 137, further comprising a step for administering said prodrug to said subject before, at the same time or after said step for administering said biocompatible matrix to said subject.

139. The use of a biocompatible matrix in the manufacture of a medicament to treat a condition, deficiency or disease of a subject, comprising:

a. encapsulating a first cell-free reaction center in a biocompatible matrix; and

b. administering said biocompatible matrix to a subject;

wherein said biocompatible matrix comprises an inorganic-based sol-gel matrix and wherein said first reaction center converts a first prodrug into a first biologically active agent in said subject.

140. The use of claim 139, wherein said biocompatible matrix comprises a silica-based sol-gel matrix.

141. The use of claim 140, wherein said first reaction center comprises one of the following: an enzyme, an antibody or a catalytic antibody.

142. The use of claim 140, wherein said first prodrug is exogenous to said subject and is administered to said subject before, at the time of, or after administration of said biocompatible matrix to said subject.

143. The use of claim 142, wherein said first prodrug is formulated in a pharmaceutically acceptable carrier and is administered to said subject on more than one occasion.

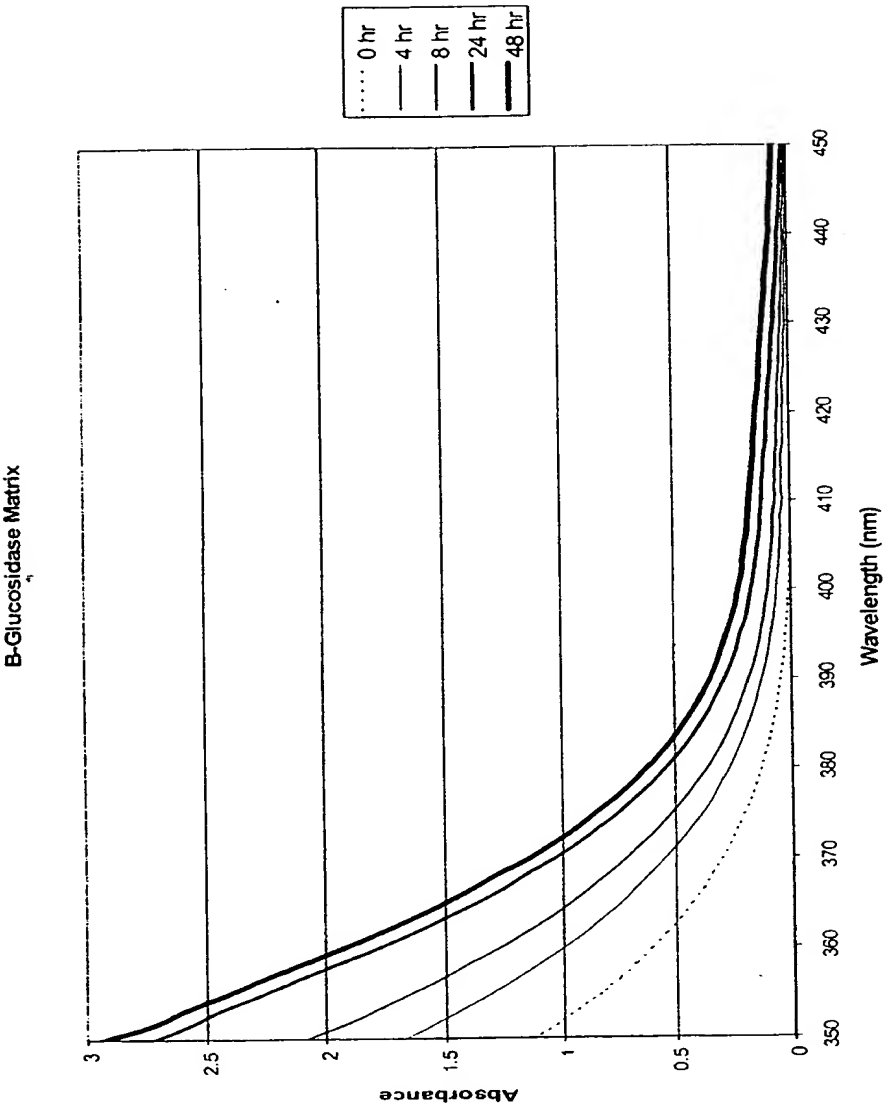


Figure 1. Enzymatic activity assay for a matrix containing β-Glucosidase.

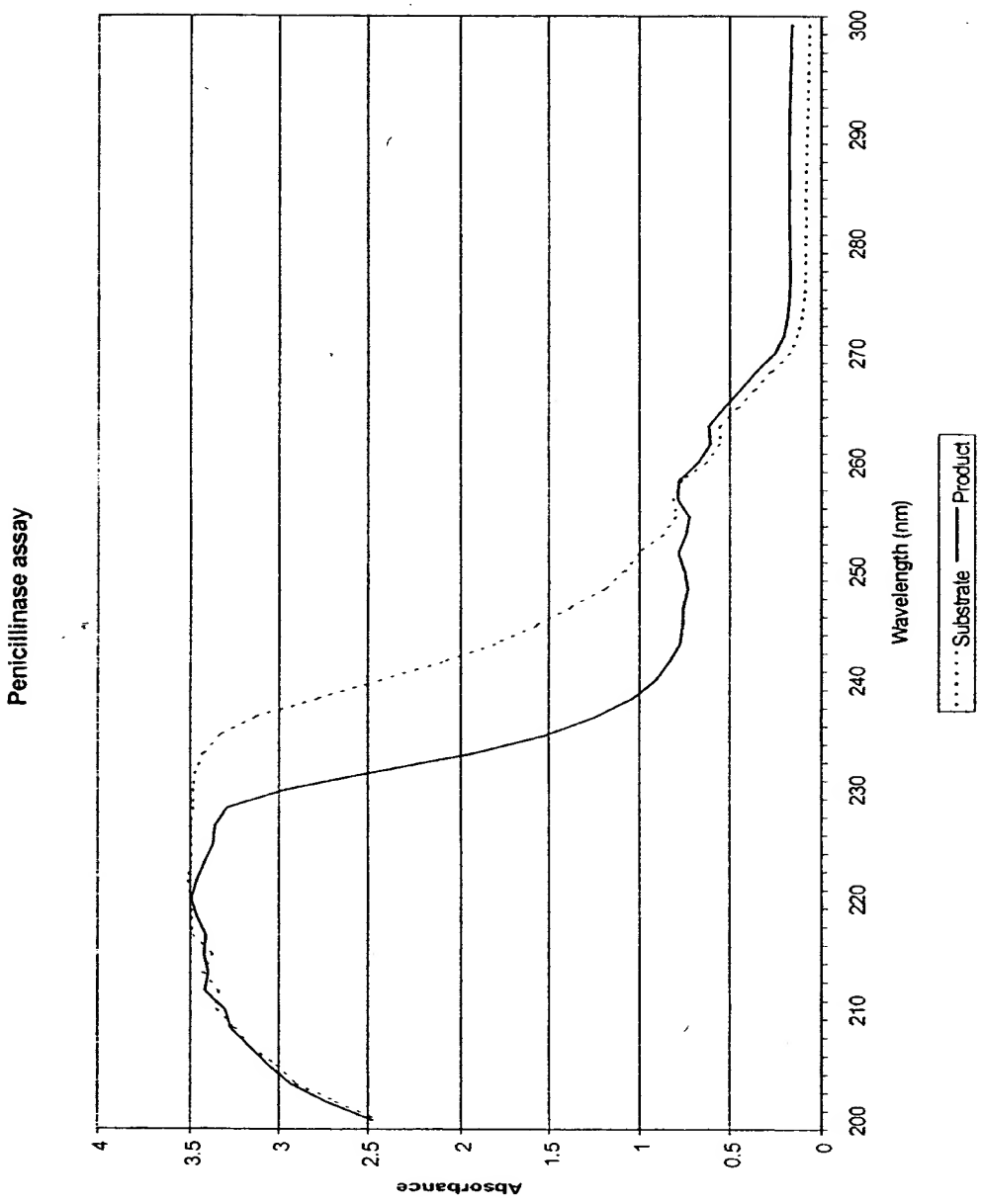


Figure 2. Substrate and product spectra for penicillinase assay.

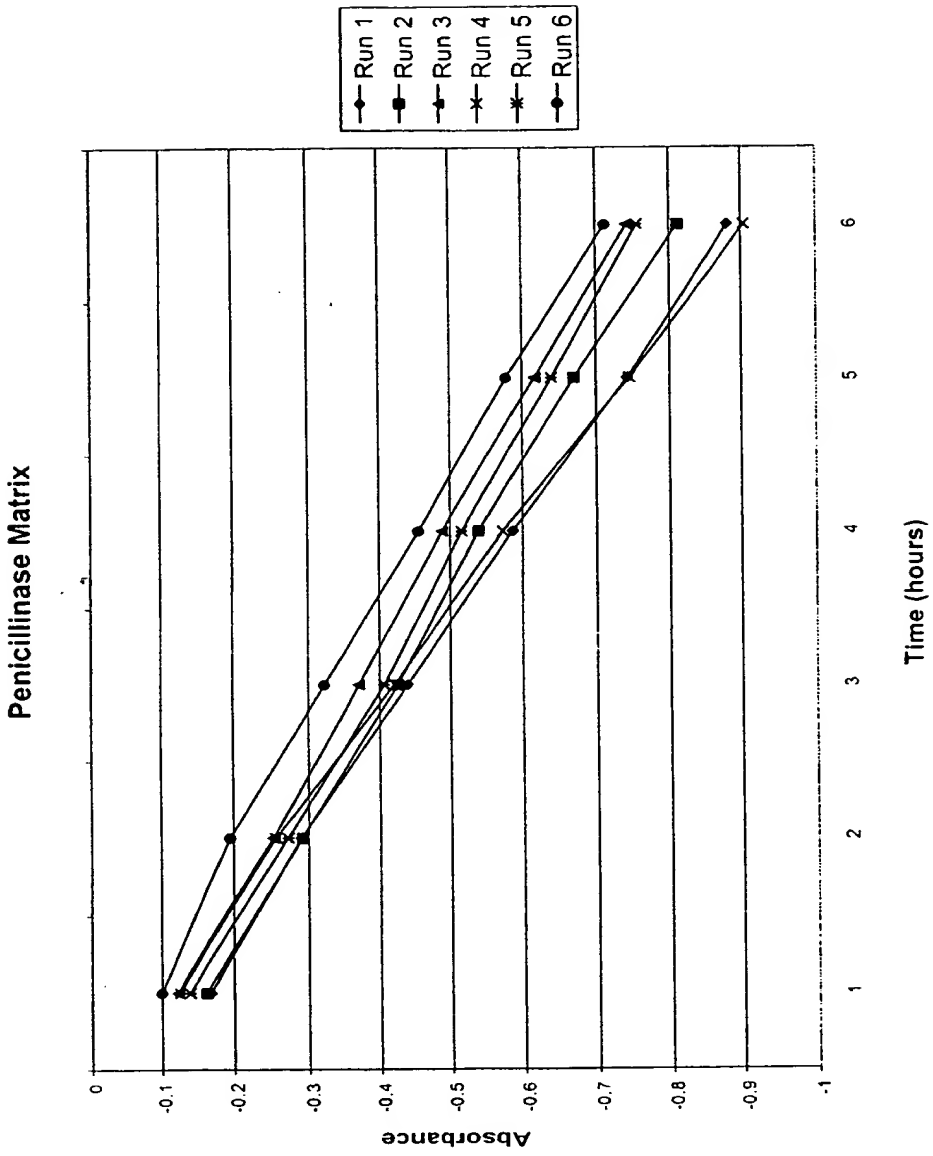


Figure 3(a). Penicillinase activity assays showing (a) multiple assays of a single matrix and (b) a single assay performed on each of five matrices from one batch preparation.

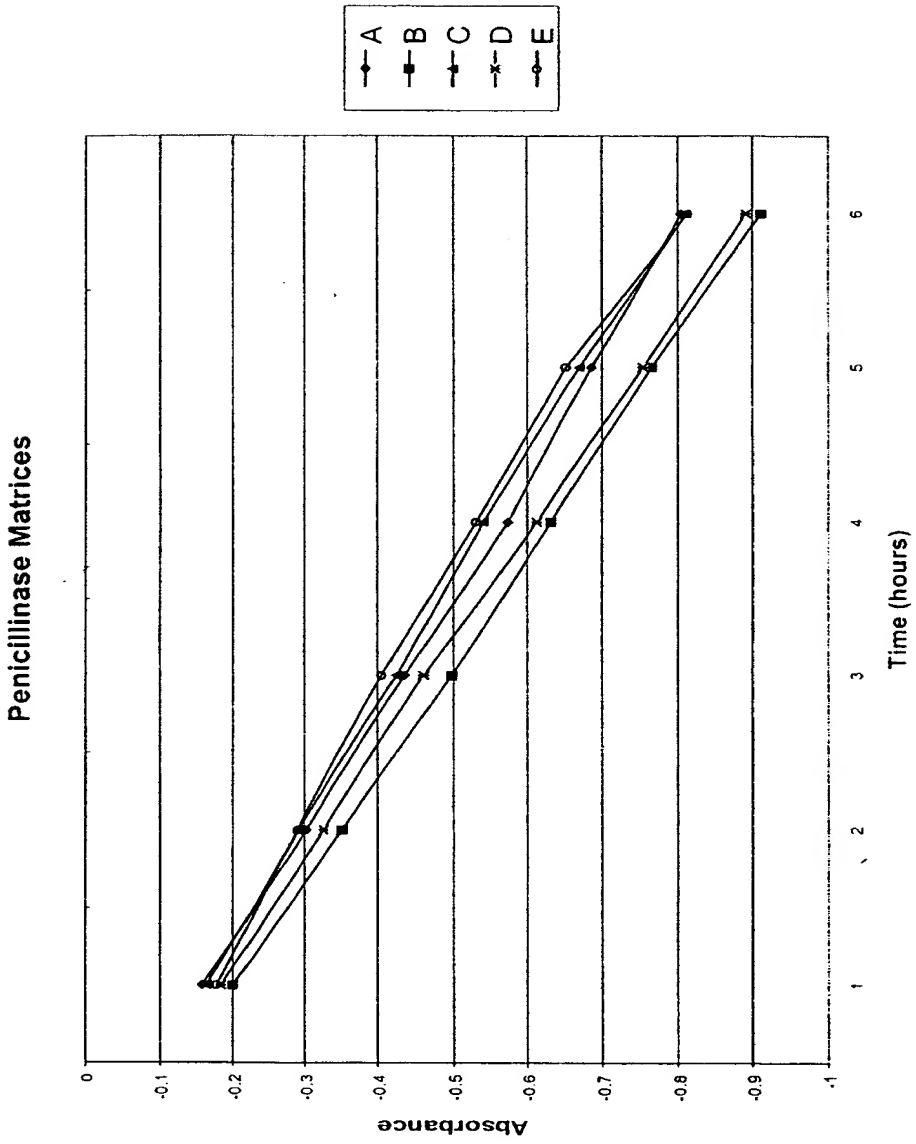


Figure 3(b). See the legend directly above.

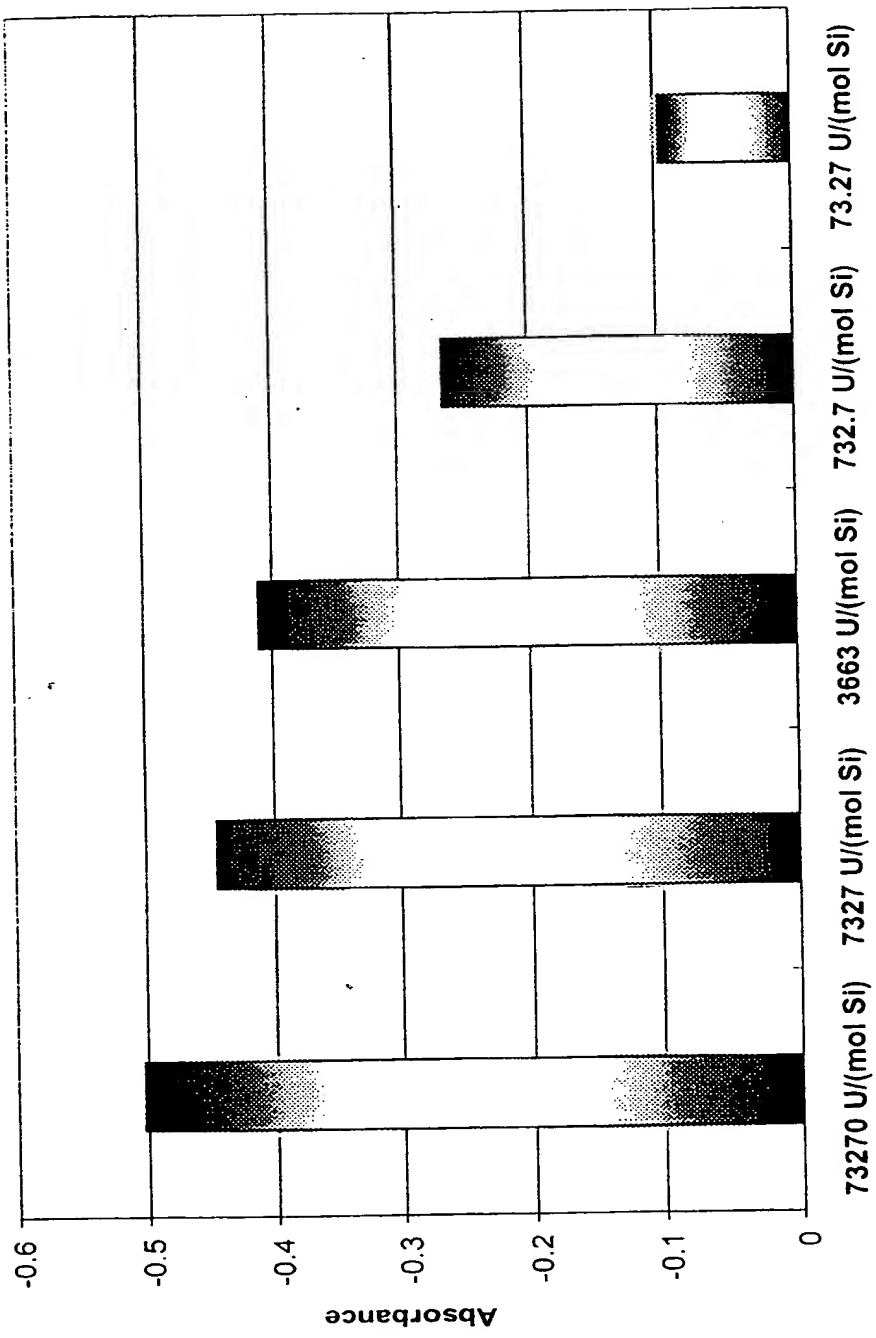


Figure 4. Change in absorbance at three hours as a function of the enzyme concentration added to the matrix during preparation.

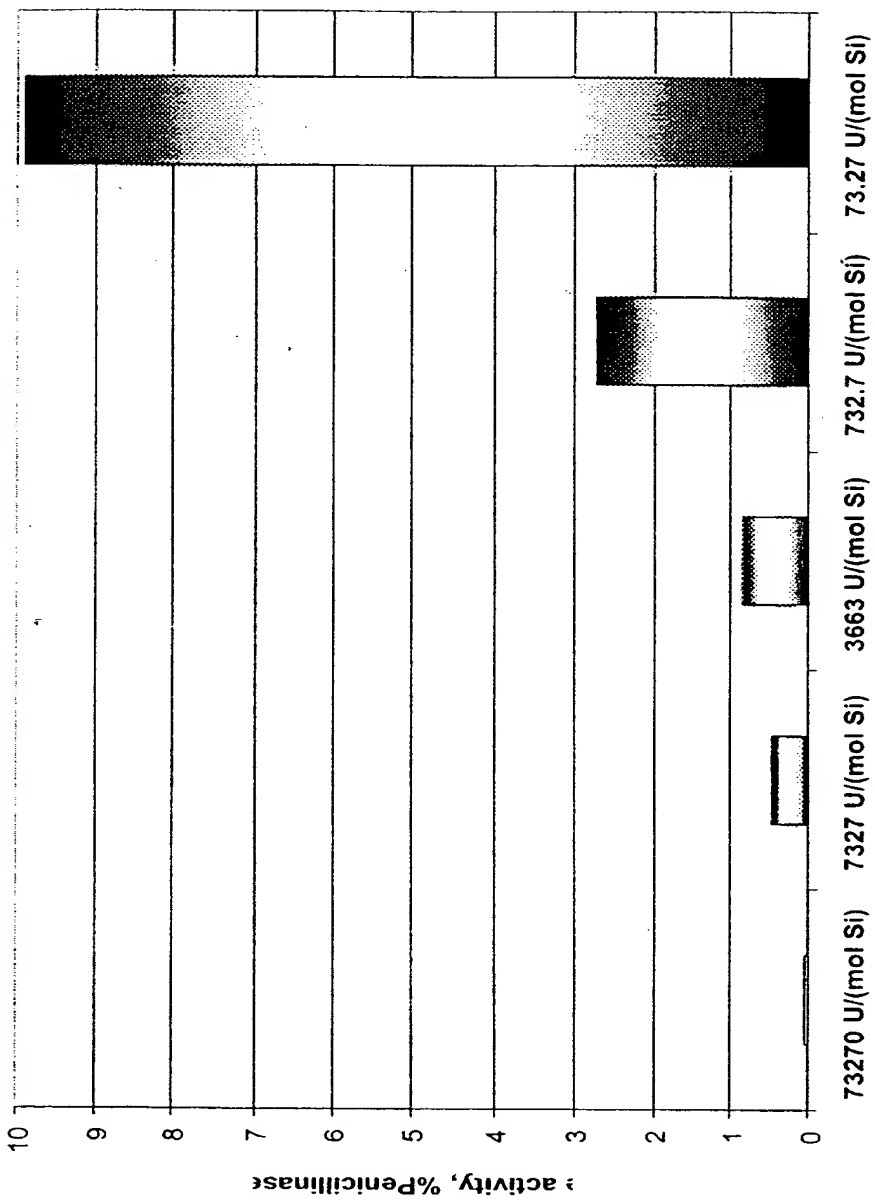


Figure 5. Yield of immobilized enzyme in penicillinase-containing sol-gel matrices. (Observed activity was calculated as the percentage of enzyme activity used in the preparation of the matrices).

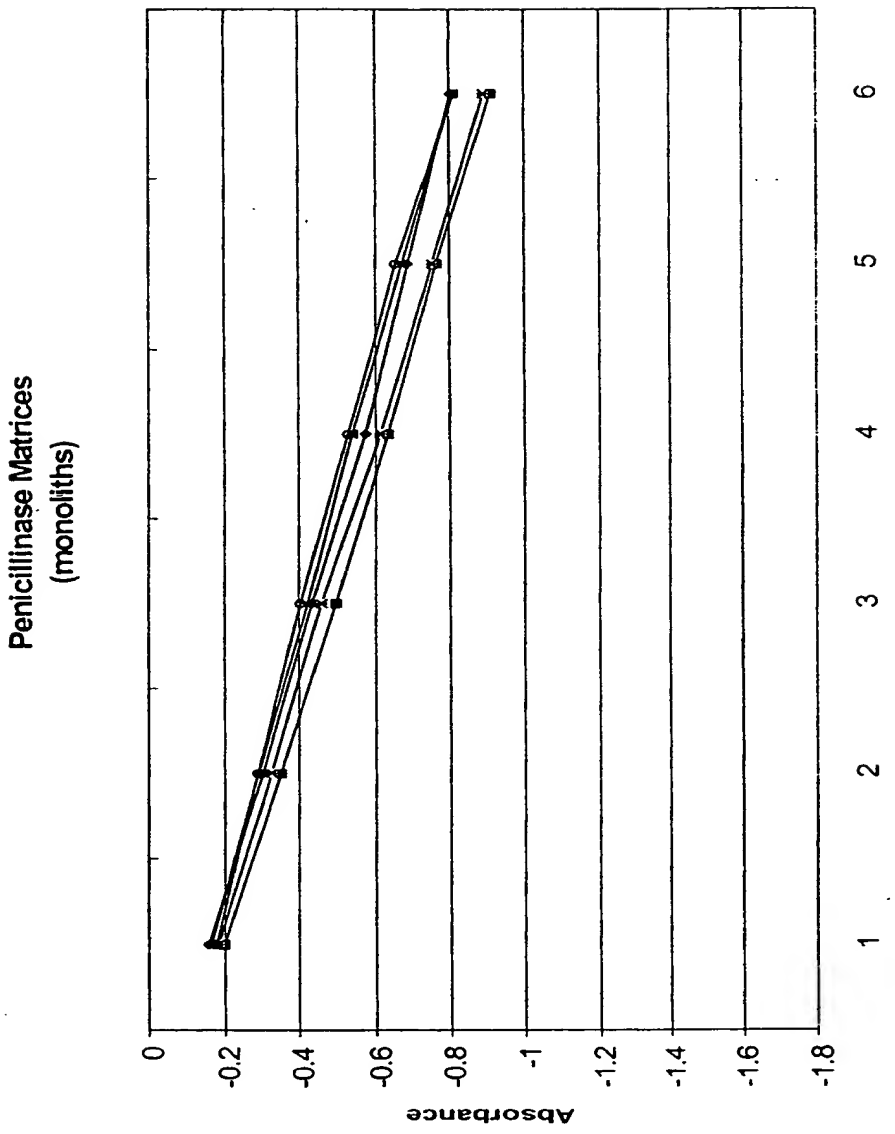


Figure 6(a). Activity of crushed and whole matrices containing penicillinase. Each figure shows data for five unique matrices assayed one time each.

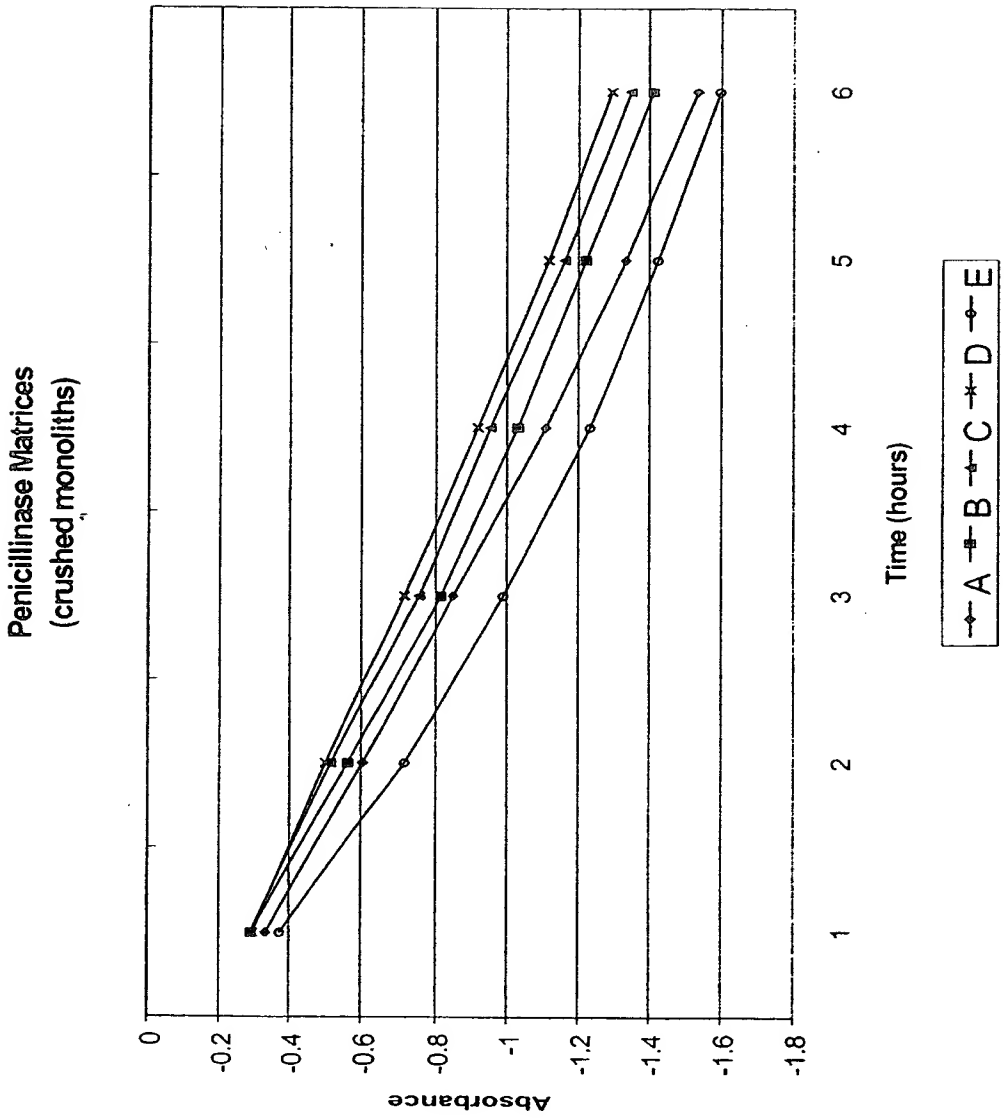


Figure 6(b). See legend directly above.

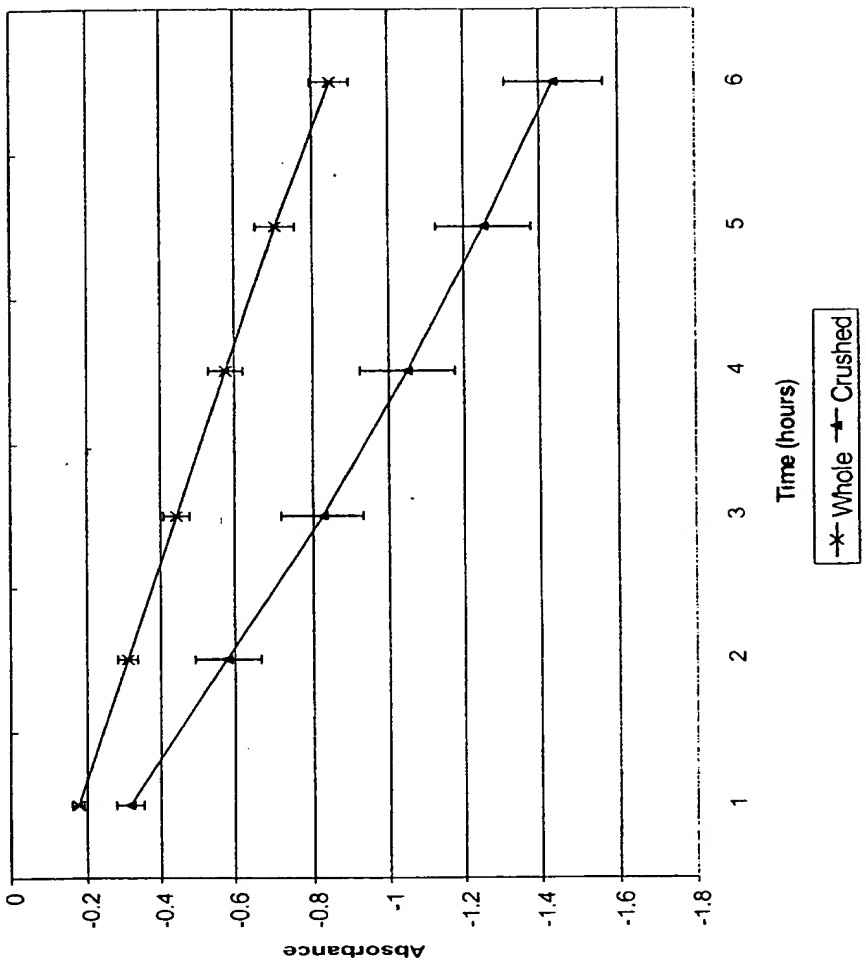


Figure 7. Penicillinase activity in whole monoliths and crushed matrices with points shown being the mean of five measurements (error bars +/- one standard deviation).

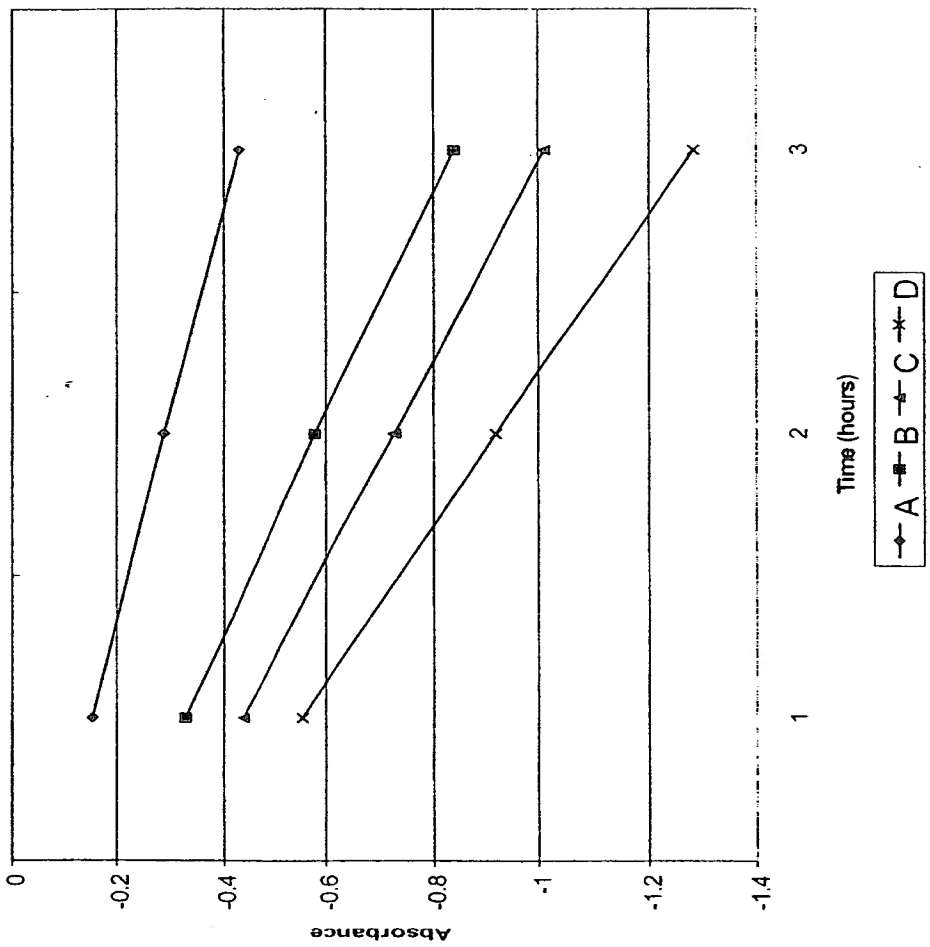


Figure 8(a). (a) Activity of penicillinase-containing matrices with varying surface areas and (b) activity as a percentage of the activity added in preparation. Surface areas corresponding to the labeling in the graphs are: A = 15.1 cm^2, B = 39.2 cm^2, C = 71.3 cm^2 and D = 135 cm^2..

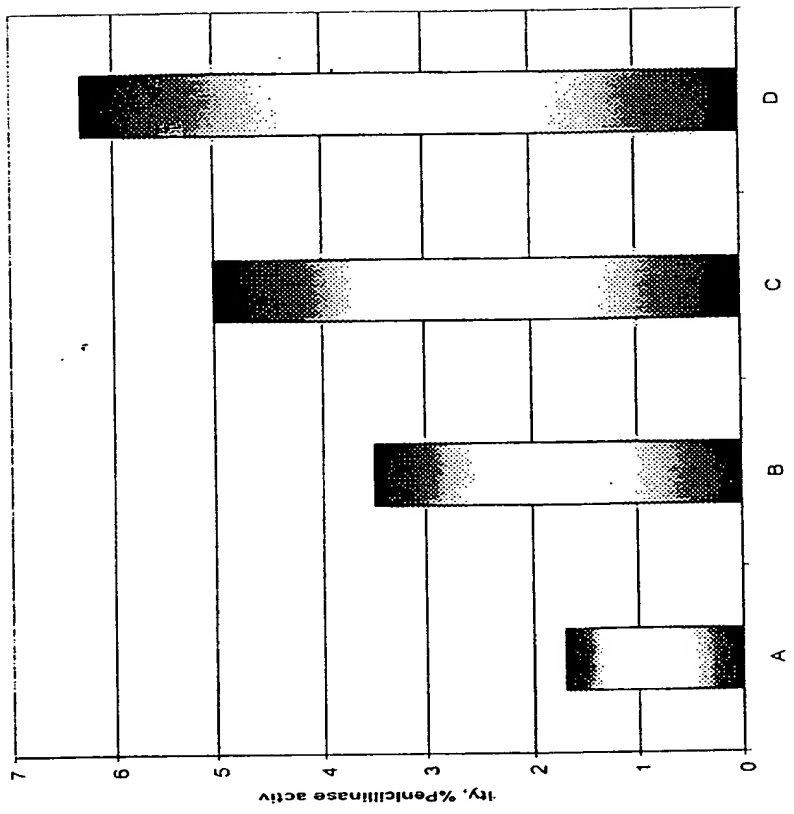


Figure 8(b). See legend directly above.

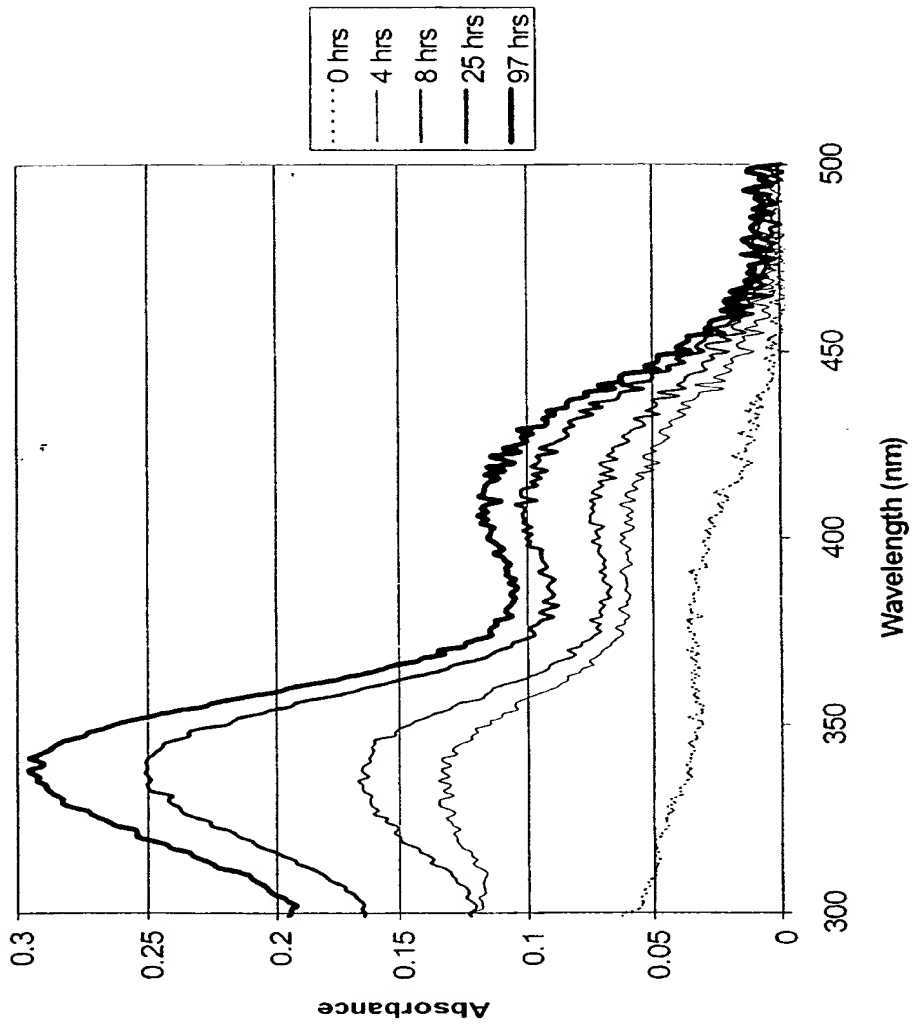


Figure 9. Tyrosine decarboxylase activity assay; elapsed time since gel cast 19.5h.

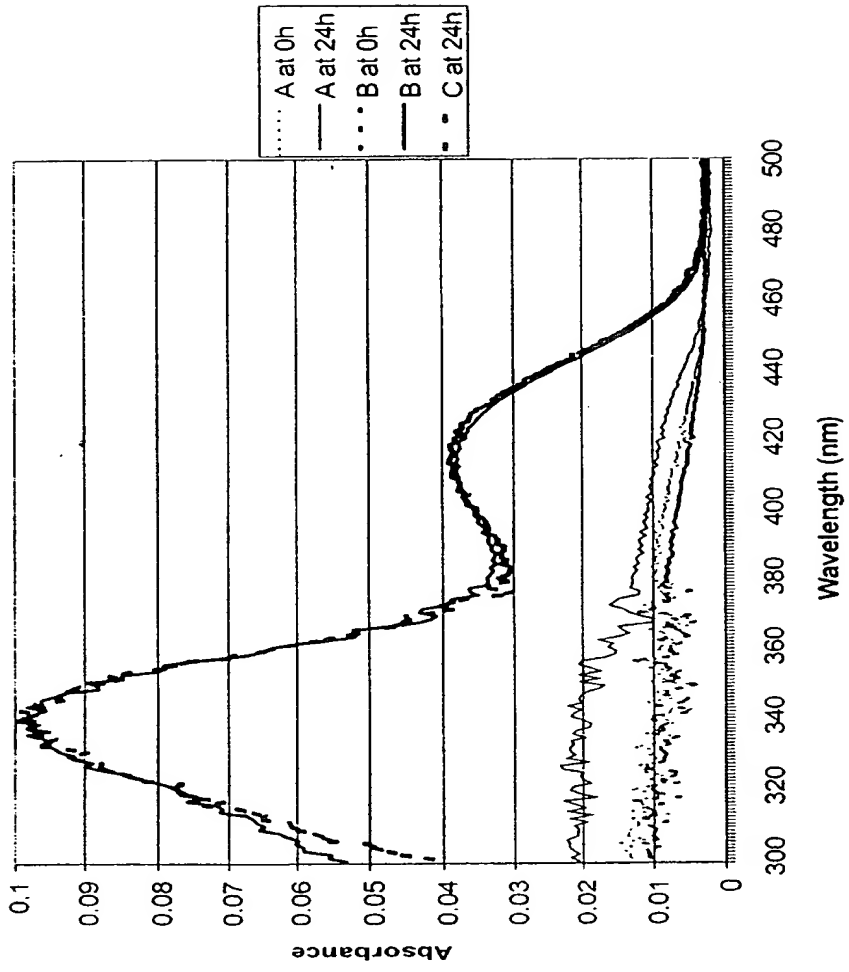


Figure 10. Tyrosine decarboxylase activity assay of two identical 16 day old matrices, A and B, with comparison to C (same matrix composition aged 19h, no cofactor present). Assay of A is performed in the absence of pyridoxal-5-phosphate (cofactor) while B is performed with cofactor present.